

Title:

Combined neuroprotective effects of propofol and dexmedetomidine on endoplasmic reticulum stress- mediated apoptosis in SH-SY5Y cells

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Running title: combined effects of propofol and dexmedetomidine

Abstract

Propofol is a short-acting intravenous anesthetic agent. Dexmedetomidine, a highly selective α_2 -adrenergic receptor agonist, shows well-known sedative effect. Both agents show cytoprotective property on a nervous system under the ischemic state. Recently, the combination of both agents is used for sedation of mechanically ventilated patients in an intensive care unit, however there are few experiment using cells regarding protective effects of the combination of propofol and dexmedetomidine. Meanwhile, intraoperative brain ischemia-reperfusion induces endoplasmic reticulum (ER)-stress mediated apoptosis. The aim of our present study is to clarify molecular details of the neuroprotection with the combination of propofol and dexmedetomidine against the thapsigargin (TG)-induced ER-stress in SH-SY5Y cells, and whether the combination assured more efficient neuroprotection.

We used TG to generate ER-stress in human neuroblastoma SH-SY5Y cells. Cells were pretreated with propofol or dexmedetomidine, or the combination of propofol and dexmedetomidine for 1 hour before co-treatment with TG for 20 hours. TG-induced apoptotic cells significantly increased $[Ca^{2+}]_i$, caspase activations, and the expressions of ER-stress biomarkers. The increase in $[Ca^{2+}]_i$ and the induction of ER-stress by TG were suppressed by pretreatment with propofol or dexmedetomidine or the combination of propofol and dexmedetomidine. The reduction of caspase activities and the ER-stress biomarkers with dexmedetomidine were inhibited by pretreatment with α_2 inhibitor. These suppressive effects of dexmedetomidine were promoted by pretreatment with cAMP inhibitor. Treatment with the combination of propofol and dexmedetomidine showed the strongest protection against TG-induced apoptosis.

These results demonstrate that the combination of propofol and dexmedetomidine at clinically relevant concentration suppressed the ER-stress induced apoptosis in neuroblastoma SH-SY5Y cells. It is suggested that the combination of propofol and dexmedetomidine within clinically relevant concentration may be used safely in patients.

Key words: neuroprotection, propofol, dexmedetomidine, ER stress-mediated apoptosis, thapsigargin

Introduction

In the 2013 Clinical Practice Guideline For Management of Pain, Agitation and Delirium in Adult Patients in Intensive Care Unit, nonbenzodiazepine-based sedation and adequate analgesia were recommended to prevent agitation and delirium. Propofol and dexmedetomidine are known that they have less agitation and delirium.

Agitation and delirium are associated with mortality and many adverse events, so we need to prevent them¹⁾. Strategies to reduce unwanted effects of anesthetic agents are important.

Propofol, a GABA agonist, is short-acting intravenous anesthetic agent and used for anesthesia and sedation but has no analgesic action. It has a preferred sedative in intensive care unit (ICU) because it offers advantages over benzodiazepines in regard to lack of accumulation, rapid onset, and rapid recovery after withdrawal. Adverse effects of propofol were reported hypotension, bradycardia, respiratory depression, and hypertriglyceridemia. Furthermore, it is known prolonged use of high dose propofol causes propofol infusion syndrome (PRIS), so prolonged use of high dose propofol should be avoided²⁾.

Propofol does not provide sufficient analgesia, so the combination with an adjuvant agent to enhance the analgesia is required. Furthermore, propofol has a dose-dependently cardiodepressive effect³⁾. For reasons of concern, propofol is usually used in combination with other drugs which have adequate analgesia⁴⁾.

Dexmedetomidine is a highly selective α_2 -adrenergic receptor agonist with both sedative and analgesic properties and devoid of respiratory depressant effect⁵⁾. Dexmedetomidine presents an alternative to the GABA agonists for ICU sedation therapy. In addition to sedation, dexmedetomidine reduces concurrent analgesic requirements while maintaining patient's arousability without compromising respiratory drive⁶⁾. Propofol and dexmedetomidine have less agitation and delirium compared with benzodiazepine, so the combination of propofol and dexmedetomidine is used for sedation in the ICU to keep spontaneous respiration and adequate sedative level. Several studies have reported that dexmedetomidine infusion significantly spares propofol requirements during the induction and the maintenance of general anesthesia⁷⁾. There are many reports that propofol has neuroprotective effects against trauma and ischemic conditions^{8, 9)}. We previously reported propofol has neuroprotective effects against ER-stress mediated apoptosis¹⁰⁾. Besides dexmedetomidine is well-proven sedative effects, evidence was indicated that dexmedetomidine exerts a cell-protective effect on nervous tissue under ischemic condition¹¹⁾. Recently, the combination of propofol and dexmedetomidine is used for sedation of mechanically ventilated patients in the ICU.

However, the relative benefits and harm between these two drugs remain controversial. Furthermore, there are few experiments using cells regarding neuroprotective effects of the combination of propofol and dexmedetomidine.

Meanwhile, intraoperative brain ischemia-reperfusion causes ER-stress to decrease glucose, energy, and ER Ca^{2+} , and then induces apoptosis. Invasive incidents such as ischemia or hypoxia induce not only changes in respiratory, circulatory, and nervous systems in the whole body but also damage to individual cells. Ischemia after surgery causes ER-stress and leads to rapid activation of the unfolded protein response (UPR)¹².

The aim of our present study is to clarify molecular details of the neuroprotection with the combination of propofol and dexmedetomidine against the thapsigargin (TG)-induced ER-stress in SH-SY5Y cells, and whether the combination assured more efficient neuroprotection.

Material and Methods

Drugs and reagents

Propofol (2,6-diisopropylphenol), dexmedetomidine (5-[(1S)-1-(2,3-dimethylphenyl)ethyl]-1H-imidazole, hydrochloride), TG, and Dulbecco's modified Eagle's medium (DMEM Ham's F-12) were purchased from Wako Co.(Osaka, Japan). Fetal bovine serum (FBS), atipamezole and 2', 5'-dideoxyadenosine(ddAdo) were purchased from Sigma-Aldrich Co. (MO, USA). Antibiotic-antimycotic (Penicillin G, Streptomycin, Amphotericin B) were obtained from life technology (USA, CA). All other chemicals used in this experiment were the purest grade commercially available.

Cell culture and drug treatment

SH-SY5Y cells (human neuroblastoma, EC-94030304) were obtained from "The European Collection of Cell Cultures" (ECACC, London, UK). Cells were cultured in DMEM Ham's F-12 containing 10 % FBS and Antibiotic-Antimycotic solution sodium and maintained in humidified atmosphere of 5 % CO₂ and 95 % air at 37 °C. For studies on effects of TG, cells were incubated in DMEM Ham's F-12 with or without TG (used as a control) for 20-hours. The SH-SY5Y cells are known to be sensitive to TG treatment¹³, and cells induced apoptosis after prolonged exposure. Furthermore, we previously reported that ER-stress was induced by 1 µM TG treatment in SH-SY5Y cells¹⁰. Cells were pretreated with propofol (1, 5, 10 µM) or dexmedetomidine (1, 10, 100, 1000 nM) or the combination of propofol and dexmedetomidine (prop+dex) for 1-hour before co-treatment with 1 µM TG for 20-hours. Atipamezole 0.3 µM (α₂-adrenoceptor inhibitor) or ddAdo 100 µM (adenylyl cyclase inhibitor) was incubated for 10 min before treatment with propofol or dexmedetomidine or the combination of prop+dex. All treatments were carried out under sterile conditions.

Measurements

caspase-3, -4 activities

Activities of caspase-3 and -4 were determined fluorometrically with respective synthetic peptide substrates.

SH-SY5Y cells were incubated with pretreated with propofol (1, 5, 10 µM) or dexmedetomidine (1, 10, 100, 1000 nM) or the combination of prop+dex for 1 hour followed by treatment with TG for 20-hours. After incubation, the cells were rinsed with cold PBS and re-suspended in chilled cell lysis buffer (Cell signaling Technology Inc., MA, USA), incubated for 10 mins on ice. Then, the supernatants were added to a reaction

buffer containing 10 μ M dithiothreitol (Medical & Biological Laboratories Co. Ltd., Aichi, Japan) and respective specific peptide substrate, after that incubated at 37 °C for 2 hours. Substrates used for caspase-3 (Kamiya Biochemical Company, Seattle, WA, USA) and caspase-4 (Bio Vision Technology Inc., SF, USA) were Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-AFC) and Leu-Glu-Ala-Asp-AFC (LEVD-AFC), respectively. AFC released by enzyme reaction was measured spectrophotometrically with an excitation wavelength of 405 nm and an emission wavelength of 505 nm with Spectra Max 340i3 (Molecular Device, CA, USA).

Supernatants were collected and protein content determined by Bio-Rad Protein Assay using bovine serum albumin (BSA) as a standard.

Assay of C/EBP homologous protein (CHOP)

After treatment with TG for 20-hours with 1-hour pretreatment with propofol (1 μ M) or dexmedetomidine (1, 10, 100 nM) or the combination of prop+dex, cells were extracted with a cold cell lysis buffer and sonicated with an ultrasonic cell disrupter. For quantitative determination of CHOP in SH-SY5Y cells lysates, ELISA kit for DNA damage inducible transcript3 (CHOP) (Usen Life Science Inc. Wuhan, China) were used.

Assay of eIF2 α (eukaryotic translation-initiation factor 2 α) phosphorylation

To examine the phosphorylation activity of eIF2 α , we used eIF2 α ELISA kit (Cell Signaling Technology, Inc. MA, USA). SH-SY5Y cells (1×10^6 cells/ml) were pretreated with propofol (1 μ M) or dexmedetomidine (1, 10, 100 nM) or the combination of prop+dex, for 1-hour followed by treatment with TG for 20-hours. Cells were fixed and blocked as directed in the manual, and incubated with anti-phospho-eIF2 α (Thr183/Tyr185) or anti-eIF2 α which are including this kit. Then they were examined spectrophotometrically at 405 nm with Spectra Max 340pc (Molecular Devices Co., CA, USA).

Assay of Cyclic AMP (cAMP) level

To examine the concentration of cAMP, we used Cyclic AMP ELISA kit (Cayman chemical company, MI, USA).

SH-SY5Y cells were incubated with pretreated with propofol (1 μ M) or dexmedetomidine (1, 10, 100 nM) or the combination of propofol and dexmedetomidine (Prop+Dex) for 1-hour followed by treatment with TG for 20-hours. After SH-SY5Y cells were pre-incubated with atipamezole, for 10-minutes before treatment with propofol or dexmedetomidine, measurement of cyclic AMP was underwent in accordance with protocol.

Intracellular free Ca^{2+} ($[Ca^{2+}]_i$) measurements by Meta Xpress Image Acquisition

Levels of $[Ca^{2+}]_i$ were analyzed using a FLIPR Calcium 5 Assay Kit (Molecular Devices, Inc., CA, USA). SH-SY5Y cells were loaded with FLIPR reagent diluted in Hank's balanced salt solution + 20 mM Hepes buffer (pH 7.4) for 1-hour at 37 °C.

After SH-SY5Y cells were incubated with FLIPR reagent, changes in the $[Ca^{2+}]_i$ were measured by monitoring the fluorescence signals of FLIPR with an excitation wavelength of 485 nm and an emission wavelength of 525 nm with the Meta Xpress Image Acquisition (Molecular Devices Inc., CA, USA).

Statistical analysis

Each measurement was repeated three times. Results are expressed as the mean \pm standard error of the mean (S.E.M.). Comparison of the effects of various treatments with those in the untreated control cells was performed with one-way ANOVA and Dunnett's as the post hoc test. Differences with p-values of less than 0.05 were considered statistically significant.

Results

Combination of propofol and dexmedetomidine reduced caspase-3 activity in ER-mediated apoptotic cells

TG-induced ER stress mediated apoptosis was detected by caspase-3 activities. (Fig. 1) In SH-SY5Y cells, neither treatment with propofol (1, 5 μ M) nor treatment with dexmedetomidine (1, 10, 100 nM) induced any significant differences in caspase-3 activity. However, when incubation with propofol (10 μ M) or dexmedetomidine (1000 nM), caspase-3 activity significantly increased compared with those in untreated SH-SY5Y cells (Fig. 1-A).

After incubation with TG for 20-hours, caspase-3 activity significantly increased compared with that of untreated control cells. When SH-SY5Y cells were pretreated with propofol (1, 5 μ M) or dexmedetomidine (1, 10, 100 nM) followed by the treatment with TG for 20-hours, the increase in caspase-3 activity was significantly suppressed. This showed that propofol or dexmedetomidine inhibited apoptosis induced by TG. However, pretreatment with dexmedetomidine at high concentration promoted ER stress-induced apoptosis.

Dexmedetomidine's effects can be inhibited by the highly specific α 2-adrenoceptor antagonist atipamezole¹⁴. When atipamezole was incubated before co-treatment with dexmedetomidine (10 nM) and TG, the caspase-3 activity significantly increased compared with TG+dex (10 nM) treated cells (Fig. 1-B). This research supported that protective effect of dexmedetomidine on ER stress-induced apoptosis was indicated through α 2-adrenoceptor.

Under TG-induced ER stress, treatment with the combination of prop+dex (10 nM) showed tendency to suppression than propofol or dexmedetomidine treatment. (Fig. 1-C).

Combination of propofol and dexmedetomidine reduced TG-induced ER stress Caspase-4 activity

Fig. 2 shows the changes in caspase-4 activity in SH-SY5Y cells. Treatment with TG resulted in significant increase in caspase-4 activity and the increase was significantly suppressed by pretreatment with propofol (1 μ M) or dexmedetomidine (1, 10, 100 nM). Similar to our previous study⁹, propofol treatment significantly decreased caspase-4 activity. When atipamezole was incubated before co-treatment with dexmedetomidine (10 nM) and TG, caspase-4 activity was significantly increased (Fig. 2-A).

Under TG-induced ER stress, treatment with the combination of prop+dex (10 nM) showed the intenser suppression than propofol or dexmedetomidine treatment (Fig. 2-

B).

CHOP level

Fig. 3 shows the changes in CHOP level in SH-SY5Y cells. Treatment with TG resulted in significant increase in CHOP level and the increase was significantly suppressed by pretreatment with propofol (1 μ M) or dexmedetomidine (1, 10, 100 nM). Under TG-induced ER stress, treatment with the combination of prop+dex showed the stronger suppression than propofol or dexmedetomidine (10 nM) treatment. When atipamezole was incubated before co-treatment with dexmedetomidine (10 nM) and TG, CHOP level was significantly increased.

eIF2 α phosphorylation

Fig.4. shows the phosphorylation of eIF2 α in SH-SY5Y cells. The eIF2 α showed significantly increased phosphorylation in TG-treated cells, which was significantly suppressed by propofol or dexmedetomidine or combination of prop+dex. Under TG-induced ER stress, treatment with the combination of prop+dex (10 nM) significantly suppressed the ER-stress, compared with propofol or dexmedetomidine (10 nM) treatment. When atipamezole was incubated before co-treatment with dexmedetomidine (10 nM) and TG, the phosphorylation of eIF2 α significantly increased, as compared with co-treatment with dexmedetomidine and TG.

Combination of propofol and dexmedetomidine decreased the intracellular cAMP

Dexmedetomidine binds to the α 2-adrenoceptor that is coupled through the Gi mechanism and inhibits adenylyl cyclase activity and downregulates cAMP formation¹⁵⁾. In the following experiments, we investigated whether the cAMP is involved in the protective effects. Pretreatment with dexmedetomidine (10, 100 nM) significantly decreased cAMP levels in TG-induced ER stress of SH-SY5Y cells. When atipamezole was incubated before co-treatment with dexmedetomidine (10 nM) and TG, the cAMP level significantly increased, as compared with co-treatment with dexmedetomidine and TG (Fig. 5). As propofol did not alter cAMP level, the results suggested that cAMP is not involved in the protective effects of propofol.

Combination of propofol and dexmedetomidine prevented TG-induced increase of [Ca²⁺]_i

We next investigated the effect of pretreatment with propofol, dexmedetomidine or the

combination of prop+dex on $[Ca^{2+}]_i$ in TG-induced ER stress of SH-SY5Y cells by Meta Xpress Images Acquisition.

$[Ca^{2+}]_i$ levels in TG-treated cells elevated and maintained high level of $[Ca^{2+}]_i$, while pretreatment with propofol or dexmedetomidine or the combination of both suppressed this continuous rising (Fig. 6). We previously described propofol treatment significantly inhibited the action of TG from the early phase, and we speculate that propofol inhibited the release of Ca^{2+} from the ER induced by TG¹⁰. Pretreatment with dexmedetomidine (10 nM) significantly suppressed cAMP levels in TG-induced ER stress of cells (Fig. 5). Pre-incubation with membrane-permeable adenylyl cyclase inhibitor ddAdo (100 μ M) showed the further suppression of $[Ca^{2+}]_i$ levels compared with co-treated with dexmedetomidine and TG. Therefore, the repression of the $[Ca^{2+}]_i$ level by dexmedetomidine has been shown to mediate a decrease in the cellular cAMP levels.

Discussion

In this study, we showed that the combination of propofol and dexmedetomidine suppressed ER-stress mediated apoptosis in SH-SY5Y cells. When SH-SY5Y cells were treated with the combination of prop+dex, ER stress-mediated apoptosis was more suppressed compared to treatment with propofol or dexmedetomidine, respectively. Especially, combination of 1 μ M propofol and 10 nM dexmedetomidine had the strongest neuroprotective effects.

Typically, patients receive 1-2.5 mg/kg propofol as an intravenous bolus injection for the induction of general anesthesia. When we use target control infusion (TCI) pump, the target plasma concentration for maintenance of propofol is usually controlled between 2.0-5.0 μ g/ml. However, propofol was extensively bound (mean: 97-98%) to the plasma protein. Fifty percent was bound to erythrocytes and 48% to serum proteins, almost exclusively to human serum albumin. Propofol free fraction in blood was 1.2-1.7% at total concentrations ranging from 0.5 to 32 μ g/ml¹⁶.

When dexmedetomidine was administrated by a loading dose of injection with 0.2-0.83 μ g/kg/h, the patients in this setting could obtain an effective dexmedetomidine concentration of 0.22-2.50 ng/ml (1.1-12.5 nM). Furthermore, the plasma dexmedetomidine concentration was moderately correlated with the administered dosage¹⁷.

Therefore, concentrations of propofol (1 μ M) and dexmedetomidine (10 nM) that we used in this study are similar to serum concentration of clinical use, it is speculated that this combination of prop (1 μ M)+dex (10 nM) may show the neuroprotective effects in clinical use.

TG decreased Ca^{2+} flow into ER from cytoplasm and reduced Ca^{2+} concentration in ER. When Ca^{2+} concentration in ER is reduced, misfolded protein is increased and causes ER-stress. The adaptive response to ER-stress is the UPR. When the UPR process works normally, overload of abnormal proteins on the ER is reduced and cell can adapt to the invasive incidents. However, upon excessively severe invasive incidents, cells cannot be adapted and apoptosis is induced via ER-stress.

ER-stress is induced at several situations such as ischemia/reperfusion, trauma and many neurodegenerative diseases, so there are many benefits to suppress ER-stress.

In our pervious study, TG treatment was suitable to generate ER-stress, so that we decided to use TG was the most appropriate for our study¹⁰.

Propofol is widely used as an intravenous anesthetic and sedative agent. The researchers treated the human glioblastoma cells with 300-500 μ M propofol and observed the increase in $[\text{Ca}^{2+}]_i$ and they reported propofol promoted apoptosis in dose-

dependent manner in 24 hours¹⁸⁾. The other researchers treated human promyelocytic leukemia cells with 150 - 250 μ M propofol and on and after 5 hours, they observed the activation of caspase-3 to induce apoptosis¹⁹⁾. However, treatment with propofol at a clinically relevant concentration showed neuroprotective effects. Propofol protects nerve, pulmonary epithelial, vascular endothelial and kidney cells ²⁰⁻²²⁾. We have previously reported that propofol (5 μ M) suppressed the ER-stress-induced apoptosis by inhibition of the Ca^{2+} release from ER¹⁰⁾.

Dexmedetomidine is a potent and highly selective agonist of the α_2 -adrenoceptors on the human brain, including clinical sedation, anesthetic-sparing effects, and analgesia²³⁾. Recent study indicated that dexmedetomidine also exhibits neuroprotection against ischemic injury ²⁴⁾ and promotes survival of nervous tissue that has suffered traumatic injury²⁵⁾. However, a study in neutrophils has shown that treatment with high concentration of dexmedetomidine (100 ng/mL: 499.3 nM) accelerated apoptosis, a clinically relevant concentration of dexmedetomidine (1 ng/mL: 4.993 nM) did not affect neutrophil apoptosis²⁶⁾. In this ER stress mediated apoptosis study, treatments with dexmedetomidine (10, 100 nM) showed the neuroprotective effect. Moreover, we indicated that pretreatment with 10 nM dexmedetomidine significantly decreased the TG-stimulated $[\text{Ca}^{2+}]_i$ elevation. Pre-incubation with membrane-permeable adenylyl cyclase inhibitor ddAdo (100 μ M) and showed the further suppression of $[\text{Ca}^{2+}]_i$ levels in co-treated cells with dexmedetomidine and TG (Fig. 6). The neuroprotective properties of dexmedetomidine have largely been attributed to its agonist actions at α_2 -adrenoceptors^{27, 28)}.

The α_2 adrenoceptor is coupled to a Gi protein complex, which inhibits adenylyl cyclase, and Ca^{2+} ion channels. The result of α_2 -adrenoceptor agonist binding is reduced cAMP production and decreased $[\text{Ca}^{2+}]_i$. Under ER stress, treatment with dexmedetomidine (10, 100 nM) significantly decreased cAMP levels (Fig. 5). Agonist stimulation of postsynaptic α_2 -adrenoceptor mediates the sedative and analgesic effect ²⁹⁾. The neuroprotective properties of dexmedetomidine have been also attributed to its agonist actions at α_2 -adrenoceptors in ER stress mediated apoptosis.

In this study, treatment with the combination of prop+dex at a clinically relevant concentration showed the strongest neuroprotective effect against TG-induced ER-stress mediated apoptosis. As mentioned above, the neuroprotection of combination of prop+dex against ER stress mediated apoptosis is caused reduction of $[\text{Ca}^{2+}]_i$. That is, propofol inhibits the Ca^{2+} release from ER¹⁰⁾, and dexmedetomidine binds to α_2 -adrenoceptors and then decreases the intracellular cAMP levels.

Dexmedetomidine has imidazole derivative structurally and binds to not only $\alpha 2$ -adrenoceptor but also imidazoline receptor³⁰). Therefore, it is necessary to demonstrate whether the neuroprotection of dexmedetomidine mediates an imidazoline receptor as well as $\alpha 2$ -adrenoceptor. Further detailed study is required to determine how the neuroprotection occurs in the combination of propofol and dexmedetomidine.

Combination of these two drugs is useable because we can reduce the dose of propofol, which has respiratory suppression, and may cause PRIS and keep adequate sedation level. We cannot apply this result to human without further study in vitro, in the future combination of propofol and dexmedetomidine is usable to protect nerve tissue under the situation which causes ER-stress such as ischemia/ reperfusion, trauma and neurodegenerative diseases.

Conclusion

We have found that the combination of propofol and dexmedetomidine has a strong protective effect on ER-stress mediated apoptosis in SH-SY5Y cells. The neuroprotection by combination is caused by the facts that propofol inhibits the Ca^{2+} release from ER and dexmedetomidine binds to $\alpha 2$ -adrenoceptors and then decreases the cAMP levels and $[\text{Ca}^{2+}]_i$.

Authors' contributions

MS, MI, TO, OR and SF carried out all the experiments. MS, MI and MT designed experiments. MS and MT wrote the paper. KO directed the study. All authors read and approved the final manuscript.

List of abbreviations

Endoplasmic reticulum (ER)

Intensive care unit (ICU)

Propofol infusion syndrome (PRIS)

Unfolded protein response (UPR)

Thapsigargin (TG)

2', 5'- dideoxyadenosine (ddAdo)

Fetal bovine serum (FBS)

C/EBP homologous protein (CHOP)

Cyclic AMP (cAMP)

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. Measurement of caspase-3 activity

Caspase-3 activities were measured using the peptide substrate (DEVD-AFC).

(A) Comparison of control cells with propofol (1, 5, 10 μ M) or dexmedetomidine (1, 10, 100, 1000 nM) on caspase-3 activity without TG in SH-SY5Y cells.

(B) The effect of propofol (1, 5, 10 μ M) and dexmedetomidine (1, 10, 100, 1000 nM) on caspase-3 activity in TG-treated apoptotic SH-SY5Y cells was examined. Atipamezole (α 2 inhibitor) was incubated before co-treatment with dexmedetomidine (10 nM) and TG (1 μ M).

(C) Pretreatment with combination of prop(1 μ M)+dex(1, 10, 100 nM) before TG-induced ER stress. Each value represents the mean \pm S.E.M. of 11–59 samples.

a: statistical difference calculated versus 1 μ M TG-treated cells ($p < 0.05$)

b: statistical difference calculated versus 1 μ M TG+1 μ M propofol treated cells ($p < 0.05$)

c: statistical difference calculated versus 1 μ M TG+1000 nM dexmedetomidine treated cells ($p < 0.05$)

d: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

#: statistical difference calculated versus control cells ($p < 0.05$)

Figure 2. Measurement of caspase-4 activity

Caspase-4 activities were measured using the peptide substrate (LEVD-AFC).

(A) The effect of propofol (1, 5, 10 μ M) and dexmedetomidine (1, 10, 100, 1000 nM) on caspase-4 activity in TG-treated apoptotic SH-SY5Y cells was examined. Atipamezole (α 2 inhibitor) was incubated before co-treatment with dexmedetomidine (10 nM) and TG (1 μ M).

(B) Pretreatment with combination of prop(1 μ M)+dex(1, 10, 100 nM) before TG-induced ER stress. Each value represents the mean \pm S.E.M. of 9–63 samples.

a: statistical difference calculated versus 1 μ M TG-treated cells ($p < 0.05$)

b: statistical difference calculated versus 1 μ M TG+1 μ M propofol treated cells ($p < 0.05$)

c: statistical difference calculated versus 1 μ M TG+1000 nM dexmedetomidine treated cells ($p < 0.05$)

d: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

Figure 3. Detection of CHOP

CHOP level was examined using ELISA kit. The effect of propofol (1 μ M) and dexmedetomidine (1, 10, 100 nM) on CHOP level in TG-treated apoptotic SH-SY5Y cells was examined. Atipamezole (α 2 inhibitor) was incubated before co-treatment with dexmedetomidine (10 nM) and TG (1 μ M). Each value represents the mean \pm S.E.M. of 6–30 samples.

a: statistical difference calculated versus 1 μ M TG-treated cells ($p < 0.05$)

b: statistical difference calculated versus 1 μ M TG+1 μ M propofol treated cells ($p < 0.05$)

c: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

Figure 4. Detection of phosphorylation of eIF2 α

Phosphorylation activity of eIF2 α was examined using ELISA kit. The effect of propofol (1 μ M) and dexmedetomidine (1, 10, 100 nM) on eIF2 α phosphorylation in TG-treated apoptotic SH-SY5Y cells was examined. Atipamezole (α 2 inhibitor) was incubated before co-treatment with dexmedetomidine (10 nM) and TG (1 μ M). Each value represents the mean \pm S.E.M. of 6–28 samples.

a: statistical difference calculated versus 1 μ M TG-treated cells ($p < 0.05$)

b: statistical difference calculated versus 1 μ M TG+1 μ M propofol treated cells ($p < 0.05$)

c: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

Figure 5. Measurement of cAMP level

cAMP level was examined using ELISA kit. The effect of propofol (1 μ M) and dexmedetomidine (1, 10, 100 nM) on cAMP level in TG-treated apoptotic SH-SY5Y cells was examined. Atipamezole (α 2 inhibitor) was incubated before co-treatment with dexmedetomidine (10 nM) and TG (1 μ M). Each value represents the mean \pm S.E.M. of 6–10 samples.

a: statistical difference calculated versus 1 μ M TG-treated cells ($p < 0.05$)

b: statistical difference calculated versus 1 μ M TG+1 μ M propofol treated cells ($p < 0.05$)

c: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

Figure 6. Measurement of [Ca²⁺]_i levels

Levels of [Ca²⁺]_i were analyzed using a FLIPR Calcium 5 Assay Kit. Alterations in [Ca²⁺]_i levels in SH-SY5Y cells treated with TG(1 μ M) or TG(1 μ M)+propofol(1 μ M) or TG(1 μ M)+dexmedetomidine(10nM) or TG(1 μ M)+ddAdo(100 μ M)+dexmedetomidine(10

nM) were measured.

(A) Photographs show alterations in $[Ca^{2+}]_i$ levels in SH-SY5Y cells. White of the brightness area shows high $[Ca^{2+}]_i$ levels in SH-SY5Y.

(B) Each value represents the mean \pm s.e.m. of 30 plates. Values are expressed as percentages in fluorescence intensity before treatment.

*: statistical difference calculated versus 1 μ M propofol, 10 nM dexmedetomidine or Prop+dex -treated cells ($p < 0.05$).

#: statistical difference calculated versus 1 μ M TG+10 nM dexmedetomidine treated cells ($p < 0.05$)

Fig1

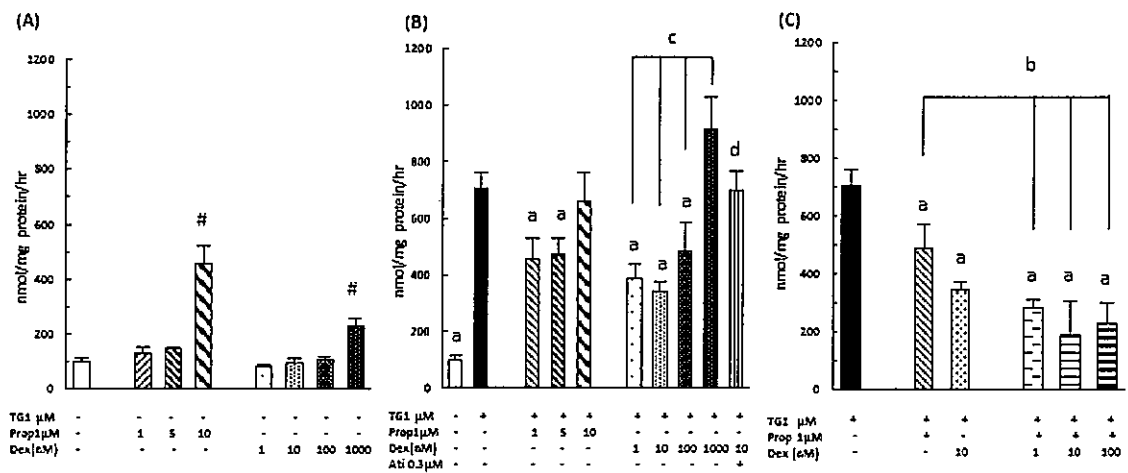


Fig2

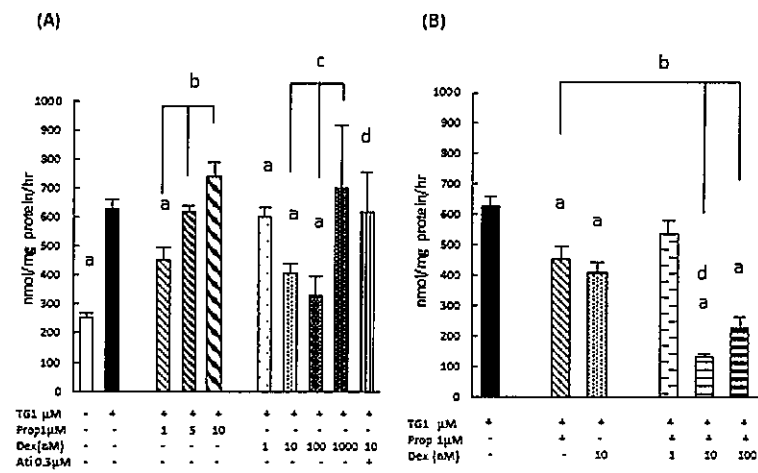


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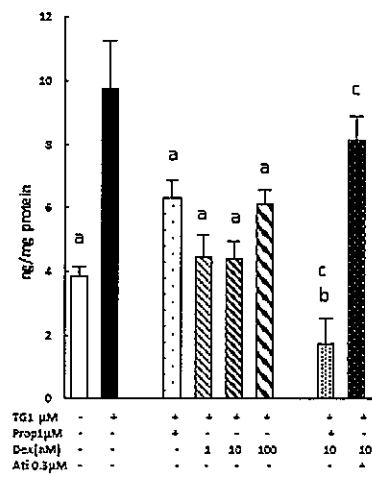


Fig4

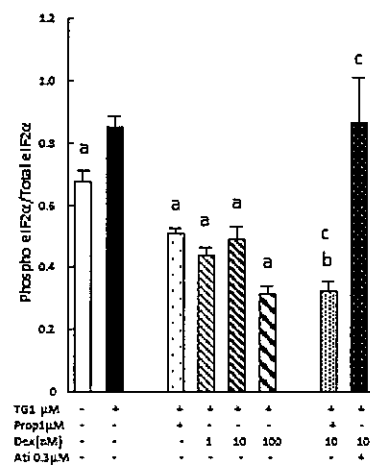


Fig5

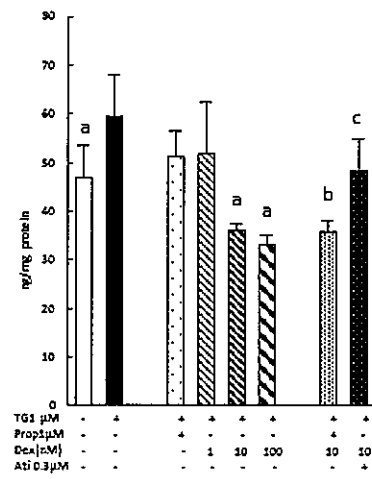


Fig6

